The effects of hypoxia on three sympatric shark species: physiological and behavioral responses

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Synopsis

Behavioral and physiological responses to hypoxia were examined in three sympatric species of sharks: bonnethead shark, *Sphyrna tiburo*, blacknose shark, *Carcharhinus acronotus*, and Florida smoothhound shark, *Mustelus norrisi*, using closed system respirometry. Sharks were exposed to normoxic and three levels of hypoxic conditions. Under normoxic conditions (5.5–6.4 mg l^{-1}), shark routine swimming speed averaged 25.5 and 31.0 cm s^{-1} for obligate ram-ventilating *S. tiburo* and *C. acronotus* respectively, and 25.0 cm s^{-1} for buccal-ventilating *M. norrisi*. Routine oxygen consumption averaged about 234.6 mg O$_2$ kg^{-1} h^{-1} for *S. tiburo*, 437.2 mg O$_2$ kg^{-1} h^{-1} for *C. acronotus*, and 161.4 mg O$_2$ kg^{-1} h^{-1} for *M. norrisi*. For ram-ventilating sharks, mouth gape averaged ~1.0 cm whereas *M. norrisi* gillbeats averaged 56.0 beats min^{-1}. Swimming speeds, mouth gape, and oxygen consumption rate of *S. tiburo* and *C. acronotus* increased to a maximum of 37–39 cm s^{-1}, 2.5–3.0 cm, and 496 and 599 mg O$_2$ kg^{-1} h^{-1} under hypoxic conditions (2.5–3.4 mg l^{-1}), respectively. *M. norrisi* decreased swimming speeds to 16 cm s^{-1} and oxygen consumption rate remained similar. Results support the hypothesis that obligate ram-ventilating sharks respond to hypoxia by increasing swimming speed and mouth gape while buccal-ventilating smoothhound sharks reduce activity.

Introduction

Oxygen availability in marine environments varies both spatially and temporally. Oxygen concentration is influenced by depth, salinity, time of day, temperature, season, and level of productivity (Perkins 1974). Areas of hypoxia are commonly found throughout shallow and open ocean areas and thus, due to this variability of the marine environment, fishes may frequently encounter hypoxic conditions.

The responses of fishes to oxygen limitation in the environment can be complex and varied. In general, fishes respond by employing a combination of behavioral and physiological styles designed to enable them to survive under hypoxic conditions. Most fish regulate oxygen uptake by increasing buccal movements to augment the flow of water over the gills (Saunders 1961, 1962). This behavioral mechanism is usually accompanied by a decrease in activity (Randall 1970). However, some fish are termed obligate ram-ventilators because they have lost the buccal pumping mechanism for gill ventilation and must maintain constant forward movement for gill ventilation and respiration (Roberts 1978).

Many shark species are obligate ram-ventilators. To increase ventilation volume under decreased oxygen levels, ram-ventilating sharks must either increase mouth gape, swimming speed, or both. Parsons & Carlson (1998) were the first to measure the response of an obligate ram-ventilating shark to hypoxia. In their study, bonnethead sharks, *Sphyrna tiburo*, increased

swimming speed, mouth gape, and oxygen consumption rate when dissolved oxygen levels decreased below $5.0 \, mg \, l^{-1}$. However, buccal-ventilating spotted dogfish, *Scyliorhinus canicula*, subjected to similar conditions were reported to decrease swimming speed and oxygen consumption rate (Metcalf & Butler 1984). These results suggest the reactions to hypoxia varies among sharks either due to different tolerance levels to hypoxia or to alternate regulatory mechanisms.

Bonnethead sharks, *S. tiburo*, blacknose sharks, *Carcharhinus acronotus*, and Florida smoothhound sharks, *Mustelus norrisi*, are commonly found in shallow coastal waters throughout the Gulf of Mexico. *S. tiburo* have been observed on shallow grass flats (1–2 m) at night during summer months where oxygen levels have been recorded as low as $2.0 \, mg \, l^{-1}$ (Carlson unpublished observation). Similarly, other shark species have been captured in the hypoxic zone that is seasonally found off the Mississippi delta (Grace & Henwood 1998). Because sharks have been observed in hypoxic areas, information concerning the behavioral and physiological responses to hypoxia is essential to understanding the environmental requirements and limitations of sharks. In addition, comparisons of results among the aforementioned studies (e.g. Metcalf & Butler 1984, Parsons & Carlson 1998) are complicated by numerous variables such as differences in temperature, acclimation state, and overall experimental design. Thus, the purpose of this study is to provide a direct comparison on the effects of hypoxia on the behavior and respiratory physiology of *S. tiburo*, *C. acronotus*, and *M. norrisi* utilizing animals subject to the same experimental conditions.

Methods

Capture and holding

Sharks (Table 1) were captured from May to June using gill nets, longlines, and rod and reel from the St. Andrew Bay System, Florida and transported to the National Marine Fisheries Service Laboratory, Panama City, Florida. At the laboratory, sharks were held for up to 30 days in outdoor, shaded, circular, 3000 liter tanks under natural photoperiod with a flow-through seawater system and fed every other day on a diet of squid or fish. All sharks used for experiments were feeding well and exhibited no signs of poor health. Prior to experimentation, sharks were not fed for 96 h to achieve a post-absorptive state.

Experimental procedure

Behavioral and physiological measurements were made in a closed, circular, respirometer (inner diameter 182 cm × depth 58 cm) constructed using a modified 1500 liter polyethylene tank. The tank was permanently sealed using silicon sealant and a plywood and Plexiglas™ lid. Plexiglas™ windows cut into the sides and top of the respirometer allowed for observations during experiments. One window in the lid was removable which allowed for placement and removal of the shark.

The experiment began by filling the respirometer with filtered, ultraviolet light sterilized, seawater at $26.1 \pm 2.2°C$. A shark was placed in the respirometer through the removable window and allowed to acclimate for 24 h. Continuous aeration was provided during acclimation. After the acclimation period, aeration ceased and the removable window was sealed to the lid. A Microelectrodes polarographic oxygen electrode (Model MI-730) connected to a Microelectrodes amplifier (Model OM-3) and Linseis strip chart recorder (Model L-4000) was calibrated at each experimental temperature and pressure prior to insertion into the respirometer. During the experiment, sharks swam continuously around the outer edge of the respirometer which allowed for water mixing. Immediately preceding and following each experiment, oxygen concentration and temperature was verified using a YSI Model 51B oxygen meter. A blank respirometer was measured for 4 h for determination of background respiration.

Table 1. Swimming speed, oxygen consumption rate, mouth gape, and gillbeat frequency in *Sphyrna tiburo*, *Carcharhinus acronotus*, and *Mustelus norrisi* under normoxic conditions. Mean wet mass, total length (± standard deviation) and sample size are also included. All other values are ± standard error.

Species	Wet mass (kg)	Total length (cm)	Swimming speed (cm s^{-1})	Oxygen consumption rate (mg O$_2$ kg^{-1} h^{-1})	Gape (cm)	Gillbeat frequency (beats min^{-1})	n
S. tiburo	1.2 ± 0.2	69.3 ± 14.1	25.5 ± 0.6	234.6 ± 16.8	1.0 ± 0.1	—	17
C. acronotus	0.7 ± 0.1	67.1 ± 7.9	31.0 ± 0.7	437.2 ± 63.5	1.1 ± 0.0	—	7
M. norrisi	1.2 ± 0.1	53.9 ± 5.4	25.0 ± 0.6	161.4 ± 6.6	—	56.0 ± 1.3	10

Replicate behavioral observations and physiological measurements were recorded every 30 min. Swimming speed (cm s^{-1}) was measured by noting the time required for the shark to pass between two points of a known distance marked on the respirometer. Gape, measured from the tip of the snout to the lower jaw, was approximated to the nearest 0.5 cm by directly comparing gape to a grid present on the window. The number of buccal movements (e.g. gillbeats) were counted for a one minute period using a stop watch. A buccal movement was defined as one synchronous expansion and contraction of the mouth.

Oxygen consumption rate (mass specific) was determined using the general equation:

$$VO_2 = \alpha\beta \, v/w,$$

where VO_2 is oxygen consumption in mg O_2 kg^{-1} h^{-1}, α is the solubility of oxygen calculated at the experimental temperature and pressure, β is the rate of change of oxygen in the respirometer, v is the volume of the respirometer and w is the wet weight of the shark. When dissolved oxygen concentrations reached between 2.5 and 3.0 mg l^{-1}, the seal was broken on the tank and the shark was removed from the respirometer, weighed (kg $\pm$ 0.1) and measured (total length, cm $\pm$ 0.1) and returned to the holding pen. In some experiments, a small group of sharks were subjected to a hypoxia-normoxia sequence. The protocol was similar to the normoxia-hypoxia sequence except no acclimation period could be provided and oxygen consumption rate could not be determined because oxygen was pumped into the respirometer.

The duration of all experiments averaged 11.5 h (range 7.0–17.6 h). Because some shark species have been shown to exhibit changes in diurnal activity patterns (Nelson & Johnson 1970, Gruber et al. 1988, Parsons & Killam 1991), experiments were conducted under constant light to eliminate the influence of environmental photoperiod. To insure that the hypoxia effect would not be confused with any endogenous activity pattern that might exist, experiments were conducted at all times of the day and night such that hypoxic conditions never consistently coincided with a particular time of day.

Statistical analysis

Behavioral and physiological responses to hypoxia were examined by grouping dissolved oxygen in four treatment levels of 3, 4, 5, and 6 ($\pm$0.5) mg l^{-1} of O_2.

The average of all replicate measurements ($\pm$s.e.) within each level was determined for each individual shark and was compared using a repeated measures 2-way analysis of variance to test for differences among oxygen levels and species (Potvin et al. 1990) (S-PLUS 2000 statistical software). The assumptions of normality and homogeneity of variance were tested using normal probability plots of residuals and plots of residuals vs. predicted values (Neter et al. 1990). If data did not meet assumptions, transformations were performed following recommendations in Zar (1984). Rejection of the null hypothesis was made at p < 0.05.

Results

Increases or decreases in shark swimming speed and oxygen consumption rate was not correlated with any time of day. Correlation coefficients were 0.093, $r^2 <$ 0.01 for bonnethead shark swimming speed with time of day and 0.01, $r^2 < 0.01$ for oxygen consumption with time of day. Correlation coefficients were -0.015, $r^2 = 0.02$ for blacknose shark swimming speed and -0.02, $r^2 < 0.01$ for oxygen consumption with time of day. Correlation coefficients were 0.05, $r^2 < 0.01$ for smoothhound shark swimming speed and 0.16, $r^2 <$ 0.01 for oxygen consumption with time of day.

Normoxia

Under normoxic conditions (5.5–6.4 mg l^{-1}), the average shark swimming speed was 25.5 $\pm$ 0.6 cm s^{-1} for *S. tiburo*, 31.0$\pm$0.7 cm s^{-1} for *C. acronotus*, and 25.0$\pm$ 0.6 cm s^{-1} for *M. norrisi* (Table 1). Oxygen consumption averaged about 234.6 $\pm$ 16.8 mg O_2 kg^{-1} h^{-1} for *S. tiburo*, 437.2$\pm$63.5 mg O_2 kg^{-1} h^{-1} for *C. acronotus*, and 161.4$\pm$6.6 mg O_2 kg h^{-1} for smoothhound sharks. For *S. tiburo* and *C. acronotus*, gape averaged $\sim$1.0 $\pm$ 0.5 cm. *M. norrisi* gillbeats averaged 56.0 $\pm$ 1.3 beats min^{-1}.

Hypoxia

Statistical differences in shark swimming speed were found among oxygen level and species. *S. tiburo* and *C. acronotus* increased swimming speed between 4.5–5.4 mg l^{-1}, reaching a maximum of 37–39 cm s^{-1} at 2.5–3.4 mg l^{-1} (Figure 1). However, *M. norrisi* decreased swimming speed in response to hypoxic conditions. A gradual decline in swimming speed was observed from 24 cm s^{-1} at 4.5–5.4 mg l^{-1} to

$16 \, \text{cm} \, \text{sec}^{-1}$ at $2.5–3.4 \, \text{mg} \, \text{l}^{-1}$. A repeated measures two-factor ANOVA detected significant differences in mean swimming speed among dissolved oxygen levels (F = 33.49, df = 3, p = 0.0001) and shark species (F = 59.71, df = 2, p = 0.0001). A significant interaction between species and dissolved oxygen was also found (F = 23.47, df = 6, p = 0.0001). Tukey-Kramer mean separation procedure found no significant differences between pairwise dissolved oxygen levels 3 and

4, and 5 and 6. Significant differences (p < 0.05) were found for all other pairwise comparisons.

Oxygen consumption for sharks was significantly different among oxygen levels (F = 14.85, df = 3, p = 0.0002) and species (F = 12.35, df = 2, p = 0.0002) and a significant interaction was observed (F = 3.86, df = 6, p = 0.002). Under hypoxic conditions, oxygen consumption remained similar throughout all treatments for *M. norrisi*. However, *S. tiburo* and *C. acronotus* significantly increased oxygen consumption at lower oxygen levels reaching a maximum of 496 and 599 $\text{mg} \, \text{O}_2 \, \text{kg}^{-1} \, \text{h}^{-1}$ at $2.5–3.4 \, \text{mg} \, \text{l}^{-1}$ for *C. acronotus* and *S. tiburo*, respectively. Post-hoc significant differences were detected only between oxygen levels 3 and 5, and 3 and 6.

Behavioral responses were found to vary among species. *S. tiburo* and *C. acronotus* reacted to hypoxia by expanding gape. Gape increased from a normoxic value of about 1 cm to a mean of 2.5–3.0 cm under hypoxic conditions (Figure 1). Increases in gape were significantly different among oxygen levels (F = 114.67, df = 3, p = 0.0001) and between *S. tiburo* and *C. acronotus* (F = 4.63, df = 1, p = 0.04). No significant interaction was found (F = 2.68, df = 3, p = 0.06). All pairwise dissolved oxygen levels were significantly different except dissolved oxygen levels 5 and 6. *M. norrisi* increased gillbeats to 61 beats min^{-1} at $3.5–4.4 \, \text{mg} \, \text{l}^{-1}$ but decreased to 55 beats min^{-1} under severe hypoxic conditions (Figure 2). No significant differences in gillbeat frequency were found among oxygen levels (F = 2.14, df = 3, p = 0.131).
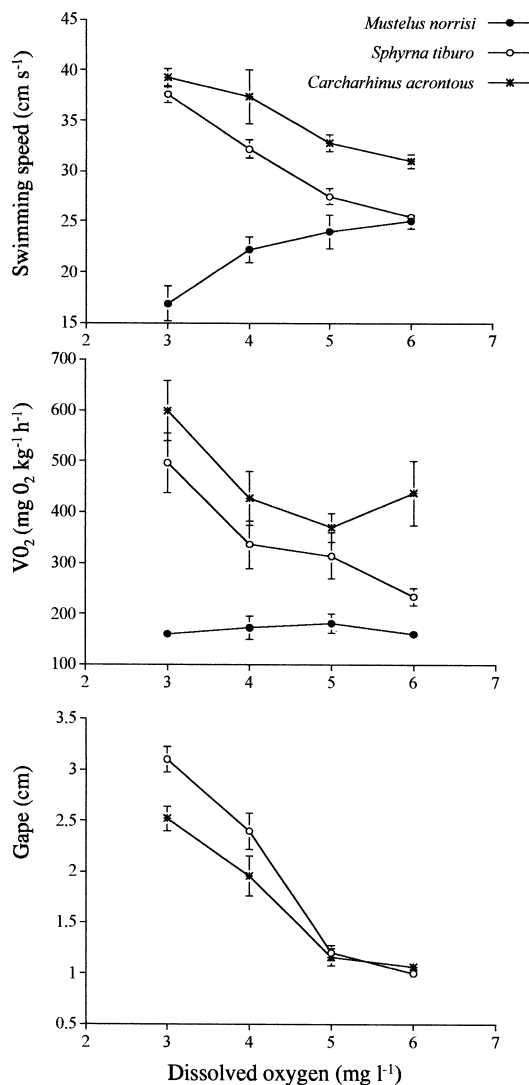

Figure 1. The effect of dissolved oxygen on *Sphyrna tiburo*, *Carcharhinus acronotus*, and *Mustelus norrisi* swimming speed, oxygen consumption rate (VO₂), and gape. Vertical bars represent ± 1 s.e.
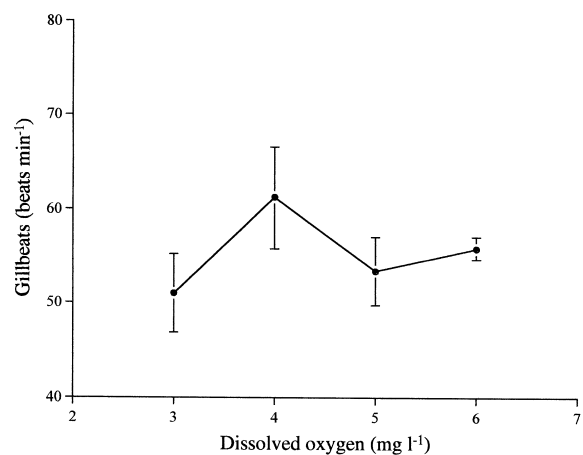

Figure 2. The effect of dissolved oxygen on *Mustelus norrisi* gillbeat frequency. Vertical bars represent ± 1 s.e.

Discussion

Oxygen availability has been generally regarded to limiting the distributions of aquatic macroorganisms (Burnett 1997). While it is usually regarded that fish avoid areas with low oxygen concentrations, evidence from this study reveals that obligate ram-ventilating sharks are able to tolerate at least moderate hypoxic conditions for long periods of time. Moreover, the behavioral and physiological mechanisms utilized by *S. tiburo* and *C. acronotus* may allow these species to exploit areas more effectively than other fish species. *S. tiburo* is a benthic predator that forages on crabs in shallow estuaries along Florida's Gulf coast (Cortés et al. 1996) primarily at night (Parsons 1987). *S. tiburo* have been observed moving from deeper areas during the day to shallow areas at night where hypoxic conditions are frequently encountered (Parsons 1987, Carlson unpublished observation). This pattern may represent an attempt to forage in hypoxic areas where other species cannot.

S. tiburo and *C. acronotus* were sensitive to hypoxia and responded by increasing swimming speed, gape and oxygen consumption in a manner similar to other ram-ventilating species. Skipjack tuna, *Katsuwonus pelamis*, dramatically increased swimming speed and mouth gape at oxygen concentrations between $3.5–4.0\,\mathrm{mg\,l^{-1}}$ (Gooding et al. 1981). Bushnell & Brill (1991) reported yellowfin tuna, *Thunnus albacares*, increased swimming speed from $52\,\mathrm{cm\,s^{-1}}$ under normoxic conditions to about $60–65\,\mathrm{cm\,s^{-1}}$ at about $90\,\mathrm{mm\,Hg}$ ($\approx 4.3\,\mathrm{mg\,l^{-1}}$).

Despite the increase in mean experimental temperature, behavioral responses were similar to that reported for *S. tiburo* at 19°C (Parsons & Carlson 1998). Since oxygen consumption rate increased with temperature, it is expected that an alteration in behavioral responses would occur to compensate for the difficulty in removing oxygen from the water at higher temperatures. However, a behavioral compensation such as an increase in gape to increase ventilation volume or decrease in swimming speed to reduce demands for oxygen did not occur with higher temperatures. This suggests that additional physiological responses could occur at higher temperatures, such as changes in the oxygen binding properties of blood, increased hematocrit, or production of hemoglobin polymorphs which would increase extraction efficiency. Some evidence for this is provided in studies on goldfish, *Carassius auratus* (Fry & Hart 1948, Houston & Cyr 1974) but

further investigation is required to test these hypotheses for sharks.

M. norrisi were also sensitive to hypoxia but reacted differently than the two ram-ventilating sharks. Free-swimming *M. norrisi*, unlike *S. tiburo* and *C. acronotus*, decreased swimming speed in response to hypoxia. Similar decreases in activity in response to hypoxia have been reported in the dogfish, *Scyliorhinus canicula* (Metcalf & Butler 1984) as well as in a variety of teleost species such as eelpout, *Zoarces viviparus* (Fisher et al. 1992); crucian carp, *Carassius carassius* (Nilsson et al. 1993); and Atlantic cod, *Gadus morhua* (Schurmann & Steffensen 1994). The reduction in swimming during prolonged hypoxic exposure could reduce energy expenditure as a considerable amount of energy may be used for activity. The energy saved may then be dedicated to additional respiratory needs such as increases in gillbeat frequency.

Increases in gillbeat frequency in response to hypoxia are a common behavioral mechanism designed to increase ventilation volume. Gillbeat increases are commonly reported in studies in a wide assortment of teleosts, such as white sucker, *Catostomus comersoni*; brown bullhead, *Ictalurus nebulosus* (Saunders 1962); European flounder, *Platichthys flesus* (Kerstens et al. 1979), and common carp, *Cyprinus carpio* (Hughes et al. 1983). Although not statistically different, *M. norrisi* increased gillbeat frequency to $61\,\mathrm{beats\,min^{-1}}$ at $4.0\,\mathrm{mg\,l^{-1}}$ but then decreased frequency under extreme hypoxic conditions. This relationship has been observed in some teleosts (Steffensen et al. 1982) as well as in buccal-ventilating sharks (Butler & Taylor 1975). The reasons for this response could be to increase respiratory amplitude under extreme hypoxic conditions, as a longer amplitude would further increase ventilation volume. Furthermore, Steffensen et al. (1982) suggested the decrease in ventilation frequency found in European flounder and European plaice, *P. platessa*, at lower dissolved oxygen levels was due to the increased energetic cost of ventilation frequency, which exceeded the benefit of oxygen delivery.

Increasing swimming speed as a mechanism for regulating respiration would appear to be metabolically costly for ram-ventilating sharks and would seem to compound the problem of obtaining sufficient oxygen in hypoxic waters. Moreover, increasing swimming speed without an accompanying increase in gape would unlikely be effective at maintaining oxygen delivery. Parsons & Carlson (1998) suggested that increasing

speed and gape may be energetically similar to other mechanisms for oxygen regulation such as increasing buccal pumping found in buccal-ventilating species. Moreover, in some mackerels and tunas swimming at speeds greater than $30\,\mathrm{cm\,s^{-1}}$, the transfer of the ventilator work load from buccal to the voluntary skeletal musculature has resulted in improved efficiency of energy expenditure for respiration and swimming (Roberts 1978).

The increases in swimming speed in response to hypoxia observed in tunas are thought to be a flight response rather than a means for increasing oxygen delivery (Bushnell & Brill 1991). In a model of gape width and swimming speed, Bushnell & Brill (op. cit.) determined that even after doubling gape and increasing swimming speed above $1.2\text{–}1.4$ body lengths $\mathrm{s^{-1}}$ oxygen delivery would not support the respiratory demands of tunas under hypoxic conditions. However, the swimming behavior observed by ram-ventilating sharks may not be a flight response but rather a mechanism for maintaining oxygen delivery. *S. tiburo* and *C. acronotus* have reduced oxygen demands with respects to tunas and increasing gape and swimming speed may provide adequate amounts of oxygen to the gills. *S. tiburo* survived hypoxic levels of $2.5\text{–}3.4\,\mathrm{mg\,l^{-1}}$ for up to $240\,\mathrm{min}$ in this study, whereas skipjack tunas tolerated low oxygen levels $(4.0\,\mathrm{mg\,l^{-1}})$ for only $20\text{–}155\,\mathrm{min}$ (Gooding et al. 1981). Moreover, Carlson (1998) determined blood oxygen content was similar at normoxic and two levels of hypoxic conditions in unrestrained free-swimming *S. tiburo*, which suggests increases in swimming speed aid in maintaining blood-oxygen levels.

We could not discern whether anaerobiosis occurred in ram-ventilating sharks during their exposure to severe hypoxia. Our experimental protocol precluded measuring a compensatory increase in oxygen consumption rate after the return to normoxia (i.e. hypoxia-normoxia experiments). Fishes that exhibit sustained or increased levels of oxygen consumption rate during hypoxia have been found to incur a oxygen debt. For example, Siberian sturgeon, *Acipenser baerii*, did not decrease oxygen consumption rate during exposure to hypoxia, but upon return to normoxia demonstrated increased plasma levels of lactate and oxygen consumption rate (Nonnotte et al. 1993).

In conclusion, sharks in this study varied in their response to hypoxia. *M. norrisi*, which have the capability to buccal pump, followed the generalized hypothesis for teleosts by reducing activity (Randall 1970) but did not demonstrate a concomitant increase in gillbeat frequency. Results also support the hypothesis that obligate-ram ventilating species respond to hypoxia by increasing swimming speed and mouth gape.

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